

Relationship of the interval between infections and the similarity of gonococcal strains in recurrent gonorrhoea

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SUMMARY Paired gonococcal isolates from 38 patients with recurrent episodes of gonorrhoea at varying intervals were examined for similarity by means of gonococcal auxotyping and susceptibility to antibiotics. A different gonococcal strain was the cause of the second infection in 53% of the patients. Longer intervals between infections were significantly associated with greater numbers of dissimilar strains as a cause of the second infection. The same strain was usually found in infections occurring within 60 days of each other whereas different strains were more likely to cause infections occurring more than 60 days apart. The isolates causing the second infection were significantly more susceptible to penicillin.

Introduction

Patients with repeated gonococcal infections are responsible for a large number of the reported cases of gonorrhoea in venereal disease clinic patients.^{1 2} The frequency of reinfection in these groups of patients has resulted in the belief that immunity to gonococcal infections may be minimal. Although both serological and humoral immune responses to *Neisseria gonorrhoeae* have been found in patients with genital infections,³⁻⁶ there is doubt that these responses are adequate to protect patients from a second infection.^{3 4} Studies of patients with repeated episodes of gonorrhoea have not examined the importance of time as a variable in the similarity of the gonococcal strain isolated from the initial and subsequent infection.

In the present study, we examined gonococci isolated from patients with repeated episodes of gonorrhoea which occurred at varying intervals. We wished to identify the frequency with which the strain of the first and second gonococcal isolate differed. Since no definitive serotyping or immunotyping system has been universally accepted for gonococcal

taxonomy, we chose auxotyping and antibiotic susceptibility testing as the methods for characterising the gonococcal isolates.

Patients and methods

BACTERIAL ISOLATES

The patients were selected from the Lexington-Fayette County Venereal Disease Clinic, Lexington, Kentucky, USA, between December 1973 and October 1977 and from the Venereal Disease Clinic of the Erasmus University Hospital (Dijkzigt), Rotterdam, the Netherlands, between September 1977 and April 1978. The former clinic serves people of primarily one county in Kentucky and the latter sailors, prostitutes, and other citizens of a large seaport. The patients were those individuals who had more than one gonococcal infection during the study period and whose gonococcal isolates could be saved. The 38 patients selected for study had infections with *N. gonorrhoeae* confirmed by cultures at separate clinic visits which took place from one to 185 weeks apart. The cultures giving positive results were from the same anatomical site in 32 of the 38 patients.

All patients were treated with antibiotics after the initial positive culture and 21 of the 38 patients had one or more cultures performed between the two infections. The patients attending the Lexington

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clinic received one of three drug regimens; aqueous procaine penicillin G 4·8 megaunits i.m. plus probenecid 1 g orally; ampicillin 3·5 g plus probenecid 1 g orally; or tetracycline 1·5 g orally followed by 0·5 g four times daily for four days. The patients at the Rotterdam clinic received amoxycillin 3 g plus probenecid 1 g orally.

The gonococci were cultured initially on modified Thayer-Martin medium.⁷ GC medium base (Difco Laboratories, Detroit, Michigan) with 1% IsoVitaleX (Baltimore Biological Laboratory, Cockeysville, Maryland) was used as the laboratory study medium. Gonococci were identified by means of colonial morphology, oxidase reaction, Gram stain, and carbohydrate reactions. The bacteria were stored at -70°C in tryptic soy broth (Difco) containing 15% glycerol. Because of the possibility that more than one gonococcal strain might infect a patient at the same time,⁸⁻¹⁰ three separate colonies were studied from the initial and final cultures from patients attending the Rotterdam clinic. Where it was possible, a second isolate from a separate site on the same patient was also examined. This isolate was usually one which had been cultured at the time of the initial visit.

ANTIBIOTIC SUSCEPTIBILITY TESTING

The antibiotic susceptibility of the gonococcal isolates to two-fold dilutions of ampicillin, penicillin, and tetracycline was tested by one of two agar plate methods as previously described.^{11 12} In addition, the isolates from the Lexington clinic were tested for susceptibility to spectinomycin.¹²

GONOCOCCAL AUXOTYPING

Gonococcal auxotyping was performed by the method of Catlin¹³ and Carifo and Catlin.¹⁴ Gonococci of known auxotype were included in each test.¹²

ANALYSIS OF DATA

Isolates from a single patient were always tested together. Gonococcal isolates were identified as separate strains if they had different auxotypes or if one or more of the antibiotic susceptibility test results differed by more than two dilutions. Analysis of the categorical data was done by the χ^2 method and the antibiotic susceptibility data were analysed by the Wilcoxon matched-pairs signed-ranks test.¹⁵

Results

The patients consisted of 14 men and 24 women from the two clinics (Rotterdam, four men and 19 women; Lexington 10 men and five women). Thirteen women

gave their occupation as prostitute, and these were all from the Rotterdam clinic. All intervening cultures gave negative results for the 21 patients who had cultures performed after antibiotic therapy for their initial infection. Cultures were not performed for the other 17 patients between their first and second infections.

GONOCOCCAL STRAINS

Fifty-three per cent (20/38) of the patients had a different gonococcal strain as the cause of their second infection. These 20 strains differed from the initial strain in the following respects: one had a different auxotype and was not tested for antibiotic susceptibility; eight, different auxotypes and antibiotic susceptibilities; nine, the same auxotype and different antibiotic susceptibilities; and two, different auxotypes and the same antibiotic susceptibilities. Of the 23 patients from the Rotterdam clinic, the same strain was found in the three colonies from each culture. However, two of these 23 patients were infected simultaneously with two strains that were identified by cultures from separate sites on the same patient during the same visit.

There was no statistically significant difference between the sexes in the number of different strains found with the second infection. Different strains were found in 57% (8/14) of the men and 50% (12/24) of the women with their second infection. Different gonococcal strains were found in 46% (6/13) of the prostitutes and 54% (6/11) of the other women, and this difference was also not statistically significant. The relationship of the interval between the two infections and the similarity of the strains is shown in the Table. Longer intervals were significantly associated with greater numbers of dissimilar strains as a cause of the second infection.

TABLE *Relationship of interval between infection and the presence of a different gonococcal strain in patients with recurrent gonorrhoea*

Interval (days)	% of strains		Total No
	Alike	Different	
<30	71·4	28·6	14
30-60	55·5	44·4	9
>60	20·0	80·0	15

$$\chi^2_1 = 7·98; P < 0·02$$

ANTIBIOTIC SUSCEPTIBILITIES

The antibiotic susceptibility of the second isolate was compared with that of the first in 37 patients. Differences in susceptibility to one or more antibiotics

were present in the paired isolates of 17 patients. There were differences in susceptibility to penicillin in 15 pairs, to ampicillin in 14 pairs, to tetracycline in 11 pairs, and to spectinomycin in seven pairs from these 17 patients. In these pairs in which there was a difference in susceptibility, the second isolate was more susceptible to penicillin in 80% (12/15) ($P < 0.05$), to ampicillin in 71% (10/14), to tetracycline in 73% (8/11), and to spectinomycin in 71% (5/7). Except for penicillin, these differences in susceptibility were not statistically significant. The distribution of the auxotypes of the first and second group of isolates was also not significantly different.

Discussion

Unlike many other infectious diseases, gonorrhoea frequently recurs in the same patient. Repeated episodes of gonorrhoea may be the result of an inadequate host defence mechanism, or possibly repeated infections may be caused by different strains, each strain producing only type-specific immunity. Since there is disagreement on the antigen or antigens which may be responsible for the latter, we chose non-immunological markers for characterising the paired isolates. Both auxotyping and antibiotic susceptibility test results are reproducible in isolates taken from the same patient.^{8 9 12 16 17}

Only a few studies have examined the characteristics of gonococci isolated from patients with repeated infections, and the influence of time on the similarity of the repeat isolates has not been emphasised. We have re-analysed data from four studies in which the interval between infections was mentioned. Tramont *et al.*¹⁸ examined isolates from five women and one man with 10 episodes of repeated gonococcal infections. The strains were characterised by an in-vitro bactericidal assay using a set of 20 typing antisera to *Neisseria meningitidis*. Repeated infections occurring within a 60-day period were predominantly with the same strain (4/5) whereas repeated infections occurring more than 60 days apart were predominantly with different strains (4/5). Silver and Darling¹⁹ examined gonococcal isolates from 20 episodes of recurrent infection after periods ranging from 4 to 119 days. The gonococci were characterised only by their susceptibility to penicillin G. In 15, the susceptibility of the second isolate was identical to that of the first; and, in five, there was only a difference of one dilution towards greater resistance. Carifo and Catlin¹⁴ examined gonococci from one man and three women, who had a total of seven repeated infections. The isolates were characterised by auxotyping and susceptibility to penicillin G. Five of the episodes occurred within 30 days. All isolates had the same auxotype and the

penicillin susceptibilities were all within one dilution. Two episodes occurred more than 130 days apart; and, in one of the two, the auxotype and penicillin susceptibility differed. In the report of Catlin and Pace,¹⁷ 25 patients had recurrent oropharyngeal gonococcal infections. The isolates were characterised by auxotyping and susceptibility to penicillin. The interval between infections was not stated for all isolates. All except one of the paired isolates had the same auxotype and penicillin susceptibility.

The finding that the second gonococcal isolate tended to be more susceptible to the antibiotics was unexpected. Susceptibility to penicillin of gonococcal isolates has been increasing in the United States²⁰ as well as in the Netherlands (Stolz, unpublished observations) since 1972, and this phenomenon has been attributed to better treatment schedules since that time.²⁰ A similar parallel was found in Greenland; the susceptibility of gonococcal strains to penicillin increased significantly between 1964 and 1967 after an intensive treatment and contact-tracing campaign.²¹ The appearance of penicillin-susceptible strains has been associated with the increased frequency of isolation of gonococci with an auxotype that was more susceptible to penicillin.²² However, the distribution of auxotypes of our initial and final isolates was not significantly different.

In the present study, longer intervals between infections were associated with a dissimilarity of the gonococcal strains causing the repeat infections. In infections occurring within a 60-day period, most of the paired isolates were the same. This difference was most striking during the first 30 days. We believe that few if any of these isolates represented treatment failures because all patients were treated with adequate antibiotic regimens,^{23 24} and all the patients who had cultures performed after treatment had one or more negative results. Infection with the same strain during the first 60-day period was probably the result of resumption of sexual contact with an untreated partner. Prostitutes were not infected more frequently with a different strain than other women. Although prostitutes undoubtedly had more sexual partners than the other women, infection could also have been acquired from an untreated regular client or friend. Other explanations for this result may be that either there were only a limited number of different gonococcal strains in the community within the study period or the methods used to study the gonococci were not sensitive enough to detect other differences between the isolates.

Thus, recurrent gonococcal infections occurring within a 60-day period are more commonly caused by the same gonococcal strain. After this interval, recurrent infections are more common with a different strain.

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